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Monounsaturated fatty acids have the potential of providing high energy density foods without the consequences of atherosclerosis and increased cancer risk associated with other high fat diets or very low fat diets. Palmitoleic acid is especially promising, and palatable food products high in this fatty acid are produced in the United States. The objectives of this study are to extend our current field of investigation of dietary use of more healthy energy dense products prepared from these natural products and high in the monounsaturated fatty acid, palmitoleic acid, for use by our armed forces in combat situations.

The study began with a 5 day run in period. Two diets, a high saturated fat diet similar in fat content to the average MRE and garrison diet (38% fat) and a diet of similar fat content (38%) but with emphasis on monounsaturated fats (22%) was fed to each of 27 individuals for 8 weeks, in random sequence in a cross over design. Thus each individual was asked to eat only study prepared meals for approximately 17 weeks.

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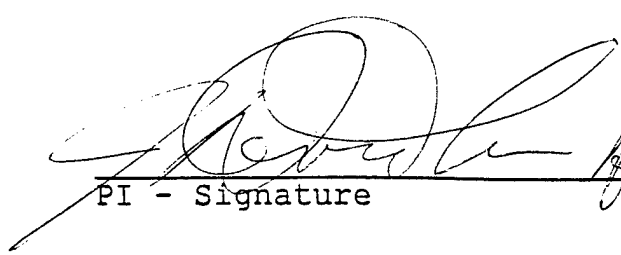
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NA In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.


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April 17, 1998
Date

**EFFECTS OF DIETS HIGH IN PALMITOLEIC ACID
ON SERUM LIPID LEVELS AND METABOLISM, Phase 2**

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EFFECTS OF DIETS HIGH IN PALMITOLEIC ACID ON SERUM LIPID LEVELS AND METABOLISM, Phase 2

I. BACKGROUND

Monounsaturated fatty acids have been demonstrated to have the potential of providing high energy density without the consequences of atherosclerosis and increased cancer risk associated with other high fat diets. Palmitoleic acid appears to be especially promising in this regard, and palatable food products high in this fatty acid are produced in the United States.

Previous animal and human studies have indicated that increased proportions of monounsaturated fats in the diet may have beneficial effects on serum total cholesterol and HDL/LDL ratios. Yamori, et. al. reported that a diet containing 1% palmitoleic acid reduced the stroke rate and increased survival in stroke-prone spontaneously hypertensive rats despite increased salt intake to a greater extent than the unsaturated fatty acid linoleic acid.¹ The Seven Countries Study² reported low rates of coronary heart disease in countries where fat intake was high, but consisted mainly of monounsaturated fatty acids. Grundy³ concluded that substitution of monounsaturated fats for saturated fats in the diet may make it unnecessary to resort to diets very low in fat to achieve optimal reduction in plasma cholesterol levels. Diets rich in monounsaturated fats can reduce total cholesterol and LDL cholesterol levels without the associated reduction of HDL cholesterol levels seen in diets very low in fat or high in polyunsaturated fat.^{4,5,6,7} Furthermore, diets high in polyunsaturated fatty acids have been found to promote tumor development after pretreatment with chemical carcinogens⁸ and to suppress the immune system^{9,10} in laboratory animals. Unlike diets high in either saturated or polyunsaturated fats which have been associated with increased cancer risk at

multiple sites, monounsaturated fat intake did not correlate with increased cancer risk at any site.¹¹

Macadamia nuts are among the natural foods known to contain a high proportion of the monounsaturated fat, palmitoleic acid. A pilot study was conducted in 1992 to determine how regular consumption of this food would affect human volunteers. Seventy-four healthy, free-living subjects, 30 to 75 years of age and having a serum cholesterol level >200 mg/dl were randomly assigned to low dose (1.6 oz/day); high dose (3.2 oz/day); or a normal diet control group for 4 weeks. After 4 weeks there were no significant changes in body weight, serum lipids, fasting glucose, laboratory measures of kidney or liver function, or blood pressure in the 3 groups. One person assigned to the high dose group reported severe diarrhea and refused to continue. This subject was included in the high dose group for statistical analysis even though he ate no nuts. For the remainder of the subjects, side effects were usually described as minimal and were elicited only on detailed questioning about gastrointestinal symptoms.

Based on this pilot study and on the results and advice of an international workshop with a group of recognized experts in the field of nutrition research organized by the researchers, a double blind randomized cross-over study with three diets was undertaken (phase one). Diet A was a "standard American diet" with 37% of calories from fat (16% saturated, 7% polyunsaturated, and 14% monounsaturated). Diet B was the American Heart Association "Prudent diet" with 30% of calories from fat (9% saturated, 7% polyunsaturated, and 14% monounsaturated). Diet C was the macadamia diet with 37% of calories from fat (9% saturated, 7% polyunsaturated, and 21% monounsaturated). Each diet was given in random sequence for four weeks. A six day run-in period preceded the first experimental diet period. Twenty-three men and nineteen

women began the study. Sixteen men and eighteen women completed the run-in period and began the experimental diets. Fifteen men and fifteen women completed the study. Those subjects who left the study left for personal reasons or for inability to adhere to the meal plan. No subject left the study for side effects. Compared to the "standard American diet", both other diets resulted in very similar and significant reductions in total and LDL cholesterol. Triglycerides increased significantly in the low fat diet, but decreased significantly in the high monounsaturated fat diet.⁷

TABLE 1. Average lipid values (S.D.) for the three dietary regimens of the Diamond Head Nutrition Research Study.

	"Typical American"	Moderate Fat Diet	Macadamia Diet
Total cholesterol	201.2 (30.4)	193.1 (34.5)*	191.3 (32.6)*
LDL cholesterol	130.4 (25.7)	124.3 (30.4)*	124.5 (29.5)*
HDL cholesterol	55.3 (7.6)	52.0 (8.2)*	52.8 (8.2)*
Triglycerides	77.5 (32.7)	83.6 (32.6)*	70.4 (26.0)*

* p < 0.05 compared to "Typical American" diet in a linear models analysis.

II. HYPOTHESIS

Substitution of the monounsaturated fatty acid, palmitoleic acid, for saturated dietary fat will result in a healthier blood lipid profile regardless of total fat intake if total calorie intake and expenditure remain balanced. This effect can be maintained over an extended period of time without appreciable adverse effects.

III. TECHNICAL OBJECTIVES (phase 2).

A group of 34 men and women age 20 to 51 was recruited to participate in a randomized study of 2 diets which was fed to each individual for 8 weeks

in random sequence in a cross over design. A 5 day run in period preceded randomization. Of the 34 men and women who initially enrolled in the project 27 completed the study. Thus each individual successfully completed in the study were asked to eat only study prepared meals for approximately 17 weeks.

Once analysis is complete (summer of 1998) it is expected that the results will show a significant improvement in the blood lipid profile and in hemostatic factors during the diet high in palmitoleic acid without significant changes in other metabolic parameters such as glucose tolerance.

IV. MILITARY SIGNIFICANCE

This study investigates further the use of energy dense dietary products prepared from natural products available in the United States and high in the monounsaturated fatty acid, palmitoleic acid, for use by the men and women of our armed forces. The development of healthier, yet acceptable energy dense dietary products for use in field and combat situations as Meals Ready to Eat (MRE) would contribute to simplified logistics while providing greater potential long term fitness. Such products might be especially applicable for Rations Lightweight (RLW) and for restricted Rations MRE's.

V. METHODS

Informed Consent

Use of human subjects was reviewed and approved by the human research committees of the University of Hawaii and other participating institutions. Informed consent was obtained from each subject after a brief screening history for exclusion criteria and before any blood tests are done or any diet given. Informed consent by proxy was not accepted.

Inclusion/Exclusion Criteria

All potential subjects were screened for exclusions including having a relatively low blood cholesterol which may be less responsive to dietary intervention. Subjects were between the ages of 18 to 55 years of age, and have had a documented serum total cholesterol between 180 and 240 mg/dl. Documentation was provided by the use of a screening cholesterol measurement. A history of allergy to tree grown nuts, diabetes mellitus or pancreatic insufficiency, fasting hypertriglyceridemia over 400 mg/dl at baseline, current pharmacologic treatment for hyperlipidemia, or an unstable medical condition of any kind as grounds to exclude the volunteer from further participation in the program. Subjects on medication for chronic medical conditions were allowed to continue if the medication is long-term and was unlikely to change in dosage or character during the course of the project (i.e. use of certain oral contraceptives). Pregnancy and breast feeding were exclusionary criteria for this study.

Baseline Evaluation

The baseline evaluation consisted of a health, dietary and family history, a brief physical examination; laboratory tests including fasting blood sugar, serum lipids, kidney, liver and thyroid function; and a serum test for pregnancy for female subjects. Report of all findings was given to each volunteer.

Recruitment and Compliance

Recruitment of subjects began August 25, 1997 and continued through September 22, 1997. Recruitment consisted of posters and flyers being placed on bulletin boards throughout the University as well as advertisements being placed in the University's student newspaper. Volunteers were directed to contact a message telephone number if they were interested in receiving more

information about the study. Approximately 400 inquiries were received by project staff during the course of the recruitment period.

Each message inquiry was followed up by research staff who contacted volunteers and provided them with a description of the project as well as the required commitments needed from volunteers to become study subjects. If the volunteer expressed continued interest in the study the telephone interviewer conducted a telephone survey questionnaire and scheduled an appointment to meet with project staff at the East West Center on the University of Hawaii campus.

The first appointment at the East West Center was to review the study protocol and receive consent to perform a screening blood draw to determine cholesterol levels. Subjects with initial screening cholesterol levels significantly under 180 were immediately eliminated for consideration. Selected subjects with cholesterol levels at or near 180 were scheduled to meet with the study nutritionist to discuss further the requirements of the study and commitment necessary for participation. Thirty five subjects (18 men, 17 women) were selected to enter the run in portion of the study. During run in, interviews continued between subjects and the study nutritionist. Ultimately one subject (a male) was excluded due to concerns regarding compliance with the study protocol. From this run in pool thirty four subjects entered the feeding sessions.

In week four one subject (a male) dropped from the study due to unforeseen difficulties in traveling to the University each day. At the end of the first feeding session and during the 60 day washout period six subjects (3 women and 3 men) dropped from the study citing personal difficulties in maintaining compliance with study protocols and compliance. Twenty seven subjects completed the studies second feeding session. Baseline information of these subjects are provided in Tables 2, 3 and 4.

**Table Two - MVNRS Baseline Information
Study Population - Demographics**

	Males (n = 13)	Females (n = 14)
Age (years)	32.5	28.4
BMI	26.4	22.5
Cholesterol	208.8	193.8
Systolic B/P	124.6	117.3
Diastolic B/P	82.0	78.2
Education:		
Undergraduate student	7	8
Bachelor's Degree	0	2
Graduate Student	6	4
Housing:		
Off Campus	9	8
On Campus	3	6
Mixed	1	0

Table Three - MVNRS Baseline Information
Study Population - Ethnicity

	Males (n = 13)	Females (n = 14)
Caucasian	3	5
Chinese	1	4
Japanese	4	1
Korean	0	2
Asian Indian	1	0
Pacific Islander	1	0
Mixed	2	2
Filipino	1	0

**Table Four - MVNRS Baseline Information
Study Population - Social Habits**

	Males (n = 13)	Females (n = 14)
Drinking:		
Never	5	6
Current	7	6
Not current	1	1
Unknown	0	1
Smoking:		
Never	12	11
Current	1	1
Not current	0	2
Using Birth Control Pill:		
No	13	11
Yes	0	3

Dietary Intervention

The dietary intervention is a controlled, double blinded crossover design feeding study of 2 dietary options lasting 8 weeks each. A run-in period of 5 days, in which a "typical American" and high mono-unsaturated fat diet was eaten, screened subjects for compliance and willingness to accept the restrictions imposed by the dietary regimens. One subject was removed from the study at run-in due to concerns about compliance.

The remaining 34 subjects were stratified by sex and randomized into the two diet groups using random numbers. Subjects received a \$600 stipend for successfully completing both diets. Partial compensation was provided on a proportional basis for those subjects withdrawing early.

The experimental diets were: 1) a high monounsaturated fat diet with 38% of calories as fat (9% saturated, 7% polyunsaturated and 22% monounsaturated fats) based on naturally occurring food products with a high content of palmitoleic acid, and 2) a "typical American" 38% fat diet (20% saturated, 7% polyunsaturated, and 11% monounsaturated fats). The percentage of calories derived from protein, and carbohydrate was held constant in each diet. Cholesterol levels were held constant for both diets.

The composition of each diet for four levels of energy intake, ranging from 1700 calories to 3000 calories, was planned by computer using the Food Processor II (ESHA Research, Salem, Oregon) and the Nutrition Data System, version 2.92 (University of Minnesota) software programs for natural and common foods. The foods in the high monounsaturated fatty acid diet were similar to those in the usual American diet with reduced portions of fatty foods and visible fats, some or all of which were replaced by products high in palmitoleic acid.

Additional calories were made in the form of "unit foods". These are in the form of 100 kcal cookies, 150 kcal muffins and 200 kcal scones. Unit foods were developed to match the nutrient profile for each diet. Subjects were allowed to eat as many of these "unit" foods as they wished in addition to their diet regimen as long as they maintained their bodyweight.

A limited amount of non-caloric caffeinated beverages were allowed (up to five cups of coffee/day). All other beverages were required to be non-caloric decaffeinated. Up to five alcoholic drinks (wine, beer, or whiskey) were allowed per week. Consumption of all foods and beverages not provided by the study were recorded in the daily dairy. It included the items and the amounts of all the foods and beverages consumed outside of those provided by the study.

Daily energy intake needed to maintain weight was estimated for each subject according to Harris-Benedict equations by an activity factor and compared to calorie intake on three-day food records completed immediately prior to the run-in period. The calculations were compared with body weight measurements and caloric levels are altered when necessary to maintain each subject's weight throughout the study.

Subjects in both diets received at least 95% of all food consumed from the study kitchen. On weekdays, subjects ate breakfast in the study dining room which was located at the East West Center. Lunches were packed in containers and given to subjects as they left breakfast. If requested these lunches were stored at the facility kitchen for pickup by subjects during lunch time. Dinners were eaten at the study dining room.

On weekends subjects ate brunch in facility dining room. Saturday dinner was declared a "free" meal. Subjects could eat a dinner prepared by

kitchen staff and taken home, or could eat a non-study prepared meal at their home or at a restaurant. The purpose of this meal was to provide a small break from the rigors of compliance so as to maintain subject morale. This non-study prepared meal had to follow guidelines provided by the study nutritionist and was recorded in detail on the daily diary. During the course of the study over 50% of the subjects elected to take home a study prepared dinner on Saturdays. On Sunday's, subjects were provided a study prepared dinner to be taken with them after they had completed their Sunday brunch.

Physical Measurements and Plebotomy

Blood pressure and weight measurement, were conducted in a small clinic located on the second floor of the East West Center. This room was set up for use exclusively by Manoa Valley Nutrition Research Staff. Weight was measured in street clothes, without heavy clothing or shoes, once during the run-in period and once a week during the study period. Illness, and medication use were reviewed weekly by interview during these measurements. Blood pressure was measured each time weight is measured. Two readings were taken in the sitting position after five minutes of rest with a standard sphygmomanometer by certified study personnel trained and standardized by protocols and methods.

Phlebotomy and processing was also conducted in these clinic. On the fifth week of each feeding session, before breakfast and after an overnight fast of over 12 hours, each subject had their blood drawn. On two successive days of the eighth week of each feeding session, before breakfast and after an

overnight fast of over twelve hours, each subject had their blood drawn. Bloods were drawn and processed using trained phlebotomist and lab technicians in the portable clinic and lab facility located on the second floor of the East West Center. All blood draws were uneventful. All used supplies and waste materials were appropriately packaged, labelled and returned to the University of Hawaii for disposal. Specimens for laboratory analysis were transported to the study repository located at Kuakini Medical Center and stored in freezer's at -70 degrees. A serum test for pregnancy was conducted monthly during the study. Urine testing was used on those monthly periods when bloods were not drawn. All tests were negative during the course of the study.

A dedicated telephone line was established for this study and the subjects were asked to call to report any problems to the study nutritionist during normal business hours using this line. The study nutritionist was also available at all times by pager. After hour contact with the study nutritionist was minimal. There were no significant problems, medical concerns, or serious adverse events.

Food and Blood Specimens

With the completion of the feeding sessions, a review has begun of mainland research laboratories methods and protocols to determine the facility most appropriate to conduct analysis of blood specimens. We anticipate that these tests will include total cholesterol, LDL cholesterol and its fractions, HDL cholesterol and its fractions, triglycerides, chylomicrons, and LP(a), fibrinogen and other tests of hemostasis as determined by the consultants, tests for short-term and long-term glucose tolerance (fasting blood glucose, fasting insulin

level, and fructosamine level), analysis of palmitoleic acid incorporation into serum lipids, and blood clotting studies.

Complete duplicate food samples of each study diet was also collected during the study period. These specimens are currently stored at the Kuakini Medical Center repository in freezers at minus 70 degrees. Mixed samples will be analyzed for levels of macronutrients and fatty acids to confirm that the composition of each diet conforms to the composition planned for the diets. A review has begun of mainland nutrition research laboratories methods and protocols to determine the facility most appropriate to conduct analysis of these food specimens.

ADDENDUM 1

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